DO MICRO AO MACRO
DESENVOLVENDO E TRANSFERINDO TECNOLOGIA

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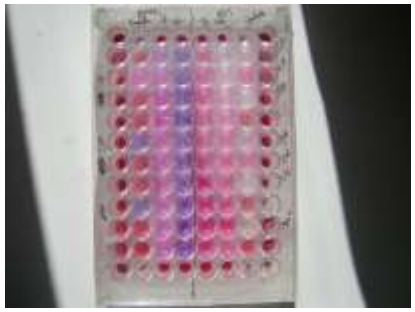
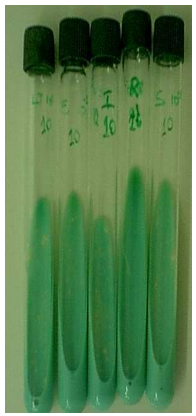
RIO GRANDE DO SUL - BRASIL

New and old methods

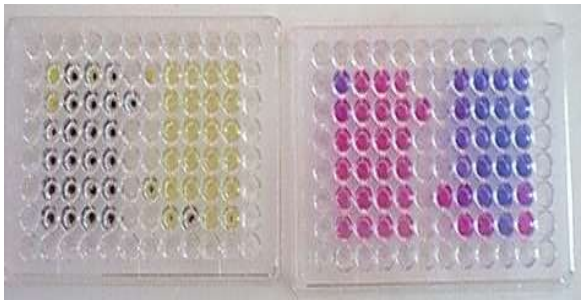
Nitratase liquid medium



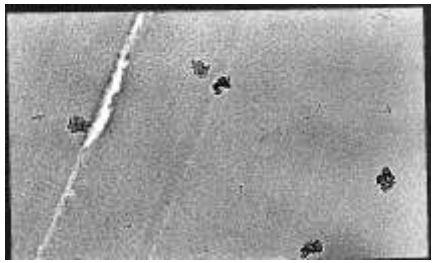
Proportion method



MTT and Resazurin

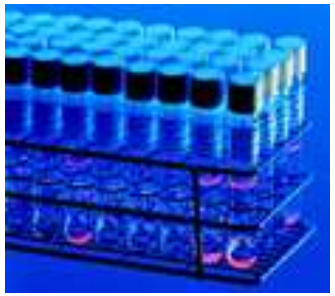


Thin layer



D29 PHAGE

MGIT



World Health Organization

NON-COMMERCIAL CULTURE AND DRUG-SUSCEPTIBILITY TESTING METHODS FOR SCREENING OF PATIENTS AT RISK OF MULTI-DRUG RESISTANT TUBERCULOSIS

- MODS: A microcolony direct method in liquid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;
- TLA: A microcolony direct method on solid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;
- CRI methods: Indirect testing methods based on the reduction of a coloured indicator added to liquid culture medium in a microtitre plate after in vitro exposure of *M. tuberculosis* strains to anti-TB drugs;
- NRA: A direct and/or indirect method based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a coloured reaction;
- Phage-based assays: Assays which uses bacteriophages to infect and detect the presence of viable *M. tuberculosis* in clinical specimens and culture isolates.

Detecção de casos

Improved of the diagnostic

INT J TUBERC LUNG DIS 12(2):218–220

SHORT COMMUNICATION

The laboratory as a tool to qualify tuberculosis diagnosis

G. Honscha,* A. Von Groll,* M. Valença,* D. F. Ramos,* K. Sanchotene,* C. J. Scaini,* M. O. Ribeiro,† P. E. A. da Silva*

* Pathology Department, Federal Foundation University of Rio Grande, Rio Grande do Sul, † Mycobacteriology Unit, Central Laboratory, Porto Alegre, Rio Grande do Sul State, Brazil

Culture (Ogawa-Kudoh) improved 30% TB cases detection

Brazilian Journal of Microbiology (2007) 38:421-423
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MICROCOLONY DETECTION IN THIN LAYER CULTURE AS AN ALTERNATIVE METHOD FOR RAPID DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* IN CLINICAL SAMPLES

Pedro Eduardo Almeida da Silva[1]*; Fernanda Wiesel[1]; Maria Marta Santos Boffo[1]; Andréa von Groll[1]; Ivo Gomes de Mattos[1]; Glória Mejia[2]; Jaime Robledo[2]

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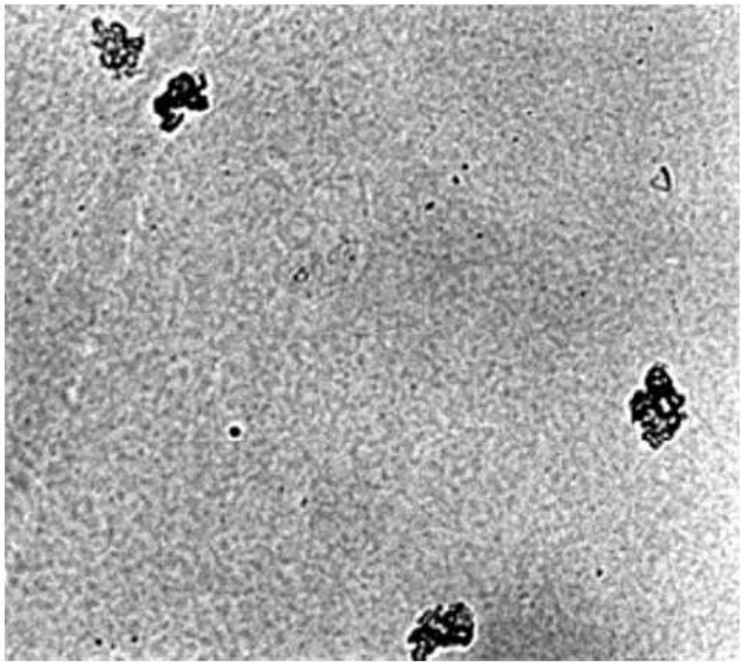

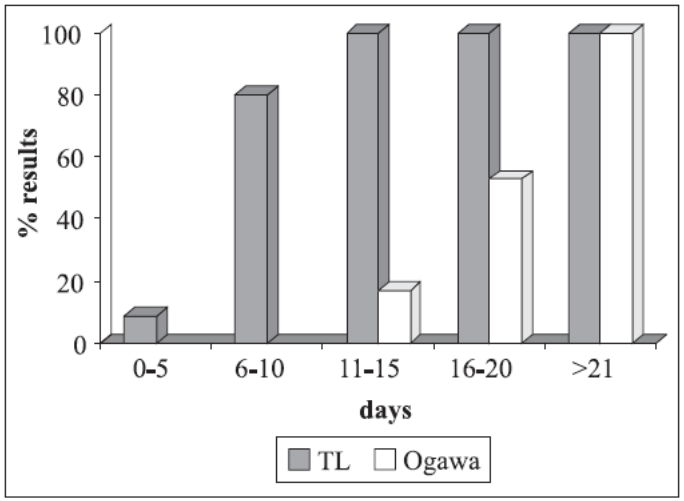

Figure 1. Cumulative percentage of *M. tuberculosis* positive results pulmonary and extra-pulmonary samples using Middlebrook 7H11 Thin Layer (TL) method and Ogawa media.

INT J TUBERC LUNG DIS 13(10):1301–1304

Thin-layer agar for detection of resistance to rifampicin, ofloxacin and kanamycin in *Mycobacterium tuberculosis* isolates

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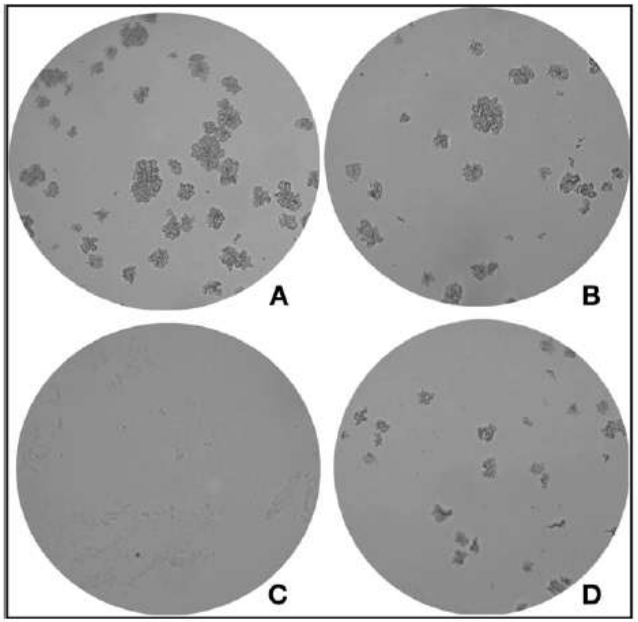


Figure Thin layer agar quadrant plate: **A**) positive growth control; **B**) rifampicin-resistant; **C**) ofloxacin-susceptible; **D**) kanamycin-resistant.

População HIV/AIDS

- 630,000 peoples with HIV/Aids in 2006
- Rio Grande do Sul showed highest Aids incidence (2008) 41,2/100,000
- Rio Grande 54,2; 49,2; 59,4; 39,4;37 in 2005, 06, 07. 08 and 2009
- HU-FURG : 1600 patients

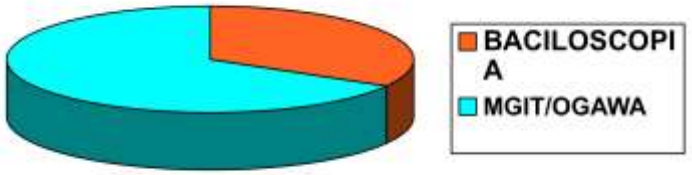
Comparação do tempo entre os Métodos

O meio de cultura MGIT teve resultados que variaram de 6 a 18 dias, em média 11 dias para o resultado final.

Já o meio convencional Ogawa-Kudoh teve variações de 22 a 42 dias, uma média de 30 dias para o resultado final.

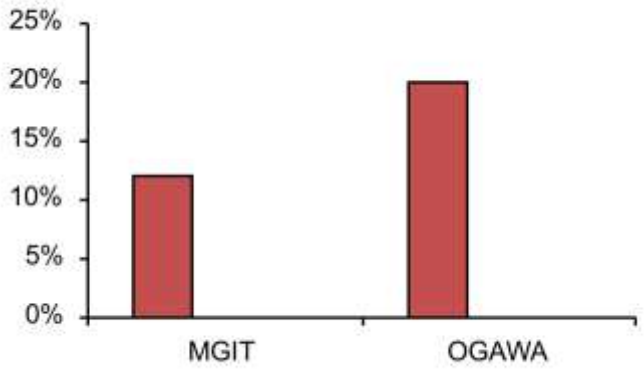
SENSIBILIDADE

- BACILOSCOPIA 43%
- MGIT 85,7%
- OGAWA 85,7%



CONTAMINAÇÃO

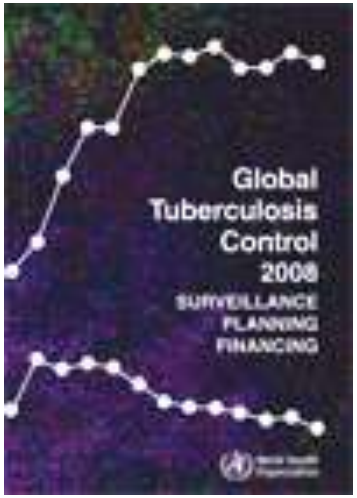
- MGIT 12%
- OGAWA 20,0%



Custos por amostra

MGIT	**R$ 9,65**
Teste Confirmatório **Tuberculose Ag MPT 64**	**R$ 10,07**
Ogawa-Kudoh **Preparado no Laboratório**	**R$ 0,30**
Ogawa-Kudoh **Industrial**	**R$ 3,60**

Cultivo e testes de sensibilidade são simples, mas….



Laboratory services[3]

Number of laboratories performing smear microscopy	4,044
Number of laboratories performing culture	193
Number of laboratories performing DST	38

Detecção de cepas resistentes

Método Canetti (Proporções)

EXECUÇÃO LABORIOSA

RESULTADOS DEMORADOS

RESISTÊNCIA DETERMINADA POR PROPORCIONALIDADE

METODOS REDOX

BAIXO CUSTO
LABORIOSO
RESULTADOS RÁPIDOS
DETERMINAÇÃO DA CMI

Da aplicação ao básico

Brazilian Journal of Microbiology (2010) 41: 300-303
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GROWTH KINETICS OF *MYCOBACTERIUM TUBERCULOSIS* MEASURED BY QUANTITATIVE RESAZURIN REDUCTION ASSAY: A TOOL FOR FITNESS STUDIES

Andrea von Groll[1], Anandi Martin[1], Françoise Portaels[1], Pedro Eduardo Almeida da Silva[2], Juan Carlos Palomino[1]

[1]Mycobacteriology Unit, Institute of Tropical Medicine Antwerp, Nationalestraat 155, B-2000, Antwerp, Belgium;
[2]Laboratorio de Micobacteriologia, Universidade Federal do Rio Grande, Rio Grande, RS, Brasil.

COMPARATIVE EVALUATION OF THE NITRATE REDUCTASE ASSAY AND THE RESAZURIN MICROTITRE ASSAY FOR DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS* AGAINST FIRST LINE ANTI-TUBERCULOSIS DRUGS

Karine O. Sanchotene[1,2]; Andrea von Groll[1,2]; Daniela Ramos[1]; Ana B. Scholante[1,2]; Gunther Honscha[1,2]; Mariana Valença[1]; Carlos J. Scaini[2]; Pedro E.A. da Silva[1,2]*

Damien Foundation - Research Project

(2010-2012)

Development of the nitrate reductase assay (NRA) for the rapid and simultaneous detection of MDR and XDR-TB in *M. tuberculosis* applied directly in sputum samples

VALIDATION OF THE NRA WITH 30 CODED STRAINS

Standard Operating Procedure (SOP)

Procedure Manual

Nitrate Reductase Assay
(NRA)

Drug susceptibility testing for Mycobacterium tuberculosis

By

Anandi MARTIN, PhD amartin@itg.be

&

Juan Carlos PALOMINO, PhD palomino@itg.be

Title: Nitrate reductase assay using sodium nitrate for rapid detection of multidrug resistant tuberculosis

Authors: Maíra Bidart de Macedo[a], Andrea Von Groll[a], Krista Fissette[b], Juan Carlos Palomino[b], Pedro Eduardo Almeida da Silva[a], Anandi Martin[b,*]

[a] Laboratório de Micobactérias, Universidade Federal do Rio Grande, Rua General Osório, sn, Rio Grande, RS, Brazil.

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KNO_3 is considered as a class of "explosive" and consequently difficult to obtain some countries such as Brazil. Additionally, $NaNO_3$ is cheaper than KNO_3 and has restriction of use.

Drug	PM	NRA-KNO_3				NRA-$NaNO_3$			
		R (n)	S (n)	Sensitivity (%)	Specificity (%)	R (n)	S (n)	Sensitivity (%)	Specificity (%)
RIF	R	72	4	95	97	73	3	96	97
	S	1	29			1	29		
INH	R	76	2	97	96	77	1	99	93
	S	1	27			2	26		

R= resistant; S=susceptible; PM=proportion method

TRANSFERÊNCIA DE TECNOLOGIA PARA O PNCT

DISTRIBUIÇÃO DA TB NO RS

TB EM PELOTAS

→ 91 pacientes detectados pelo cultivo

→ 72 incluídos na análise de perfil epidemiológico

→ 19 pacientes não foram encontrados nos registros do PMCT

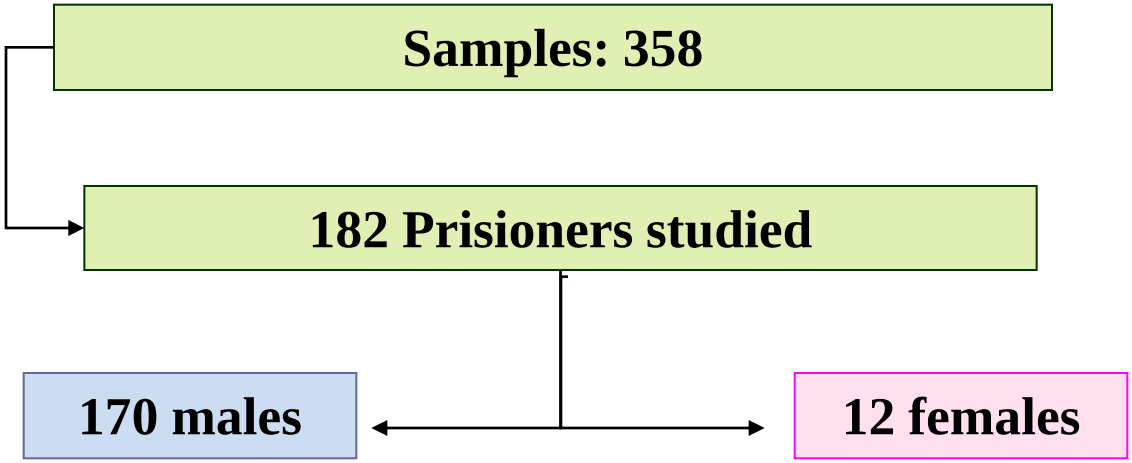
Resistência

1 caso de monorresistente (INH)

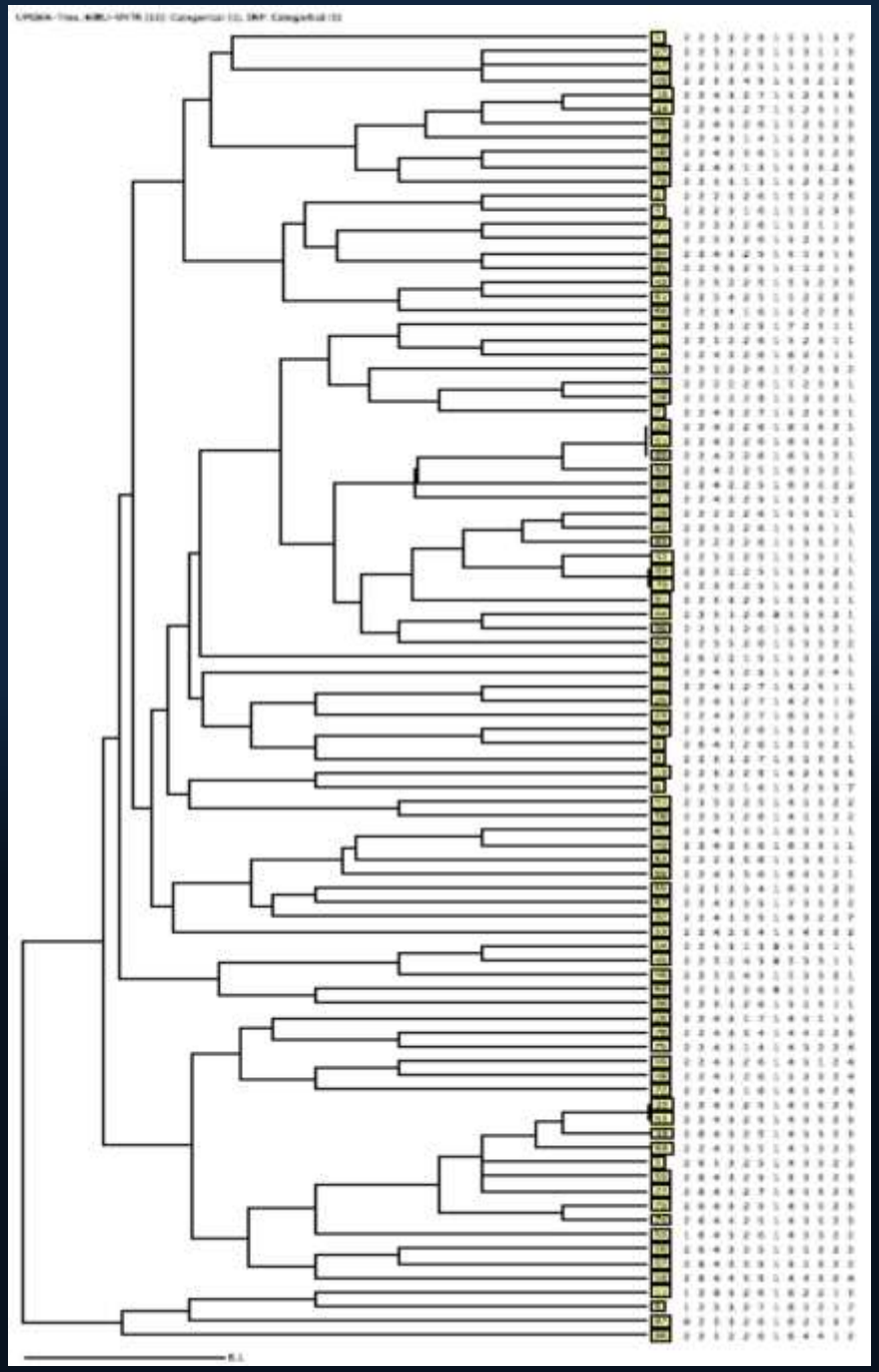
2 casos MDR (INH e RIF)

Tratamento prévio

Preliminary results in Prison of Pelotas



10 prisioners with TB (9 males and 1 female)



Os 10 isolados provenientes da penitenciária não possuem um perfil idêntico mas situaram-se próximos no dendograma, diferindo em um ou dois *loci*.